VITAMIN D THERAPY TO REDUCE BLOOD PRESSURE AND LEFT VENTRICULAR HYPERTROPHY IN RESISTANT HYPERTENSION – A RANDOMISED CONTROLLED TRIAL

Miles D Witham[1]

Sheila Ireland[2]

J Graeme Houston[3]

Stephen J Gandy[5]

Shelley Waugh[5]

Thomas M MacDonald[4]

Isla S Mackenzie[4]

Allan D Struthers[2]

Running head: Vitamin D in resistant hypertension

[1]Ageing and Health, [2]Dept of Clinical Pharmacology, [3]Dept of Clinical Imaging, [5]Hypertension Research Centre and Medicines Monitoring Unit (MEMO), University of Dundee; [5]Dept of Medical Physics, NHS Tayside.

Correspondence to: Dr Miles D Witham, Clinical Senior Lecturer in Ageing and Health, Ninewells Hospital, Dundee DD1 9SY

Tel: 01382 632436

Fax: 01382 660675

Email: m.witham@dundee.ac.uk

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Abstract

Low 25-hydroxyvitamin D levels are associated with higher prevalent blood pressure. We tested whether high-dose intermittent oral vitamin D therapy could reduce blood pressure and left ventricular mass in patients with hypertension resistant to conventional treatment. We conducted a parallel-group, double-blind, randomised placebo-controlled trial. Patients with supine office blood pressure >140/90 mmHg on 3 or more antihypertensive agents received 100,000 units oral vitamin D3 or matching placebo every 2 months. Office and 24 hour ambulatory blood pressure, glucose and cholesterol were measured at baseline, 2,4 and 6 months; left ventricular mass index was measured by cardiac magnetic resonance imaging on a subgroup at baseline and 6 months. The primary outcome was mean 24 hour ambulatory blood pressure at 6 months. 68 participants were randomised, 34 to each group. Mean age was 63 (SD 11) years, mean baseline office blood pressure was 154/84 (13/10) mmHg and mean baseline 25-hydroxyvitamin D level was 42(16) nmol/L. Treatment with vitamin D did not reduce 24 hour ambulatory blood pressure (adjusted treatment effects: systolic +3mmHg, 95%CI -4 to +11, p=0.33; diastolic -2mmHg, 95%CI -6 to +2, p=0.29); similar results were seen for office blood pressure. Left ventricular mass index was measured in a subgroup (n=25); no reduction was seen with vitamin D treatment (adjusted treatment effect +4g/m^2, 95%CI 0 to +7, p=0.04). There was no significant change in cholesterol or glucose levels. Thus six months of intermittent, high-dose oral vitamin D3 did not reduce blood pressure or left ventricular mass in patients with resistant hypertension.

Key words: resistant hypertension, vitamin D, left ventricular mass, blood pressure, randomised controlled trial

Introduction

Resistant hypertension, defined as an office systolic blood pressure of >140mmHg or diastolic blood pressure of >90mmHg despite maximally tolerated treatment with three or more antihypertensive agents, affects 10 to 30% of patients with hypertension[1]. Resistance to treatment is associated with a high incidence of cardiovascular events[2]. Resistant hypertension is also associated with a high incidence of left ventricular hypertrophy[3], itself a risk factor for cardiovascular events, arrhythmias and death[4].

Relatively few studies target resistant hypertensive patients as a discrete subgroup, and treatment algorithms are not as well defined for this group of patients as for those with more straightforward mild to moderate hypertension. Despite a wide range of antihypertensive agents now being available with multiple modes of action, treatment of resistant hypertension remains problematic, with many patients suffering from treatment-limiting side-effects[5]. Although recent interest has focussed on invasive solutions such as renal denervation therapy[6], the large burden of resistant hypertension at the population level means that inexpensive, easily applied interventions to mitigate the problem are still required.

Low vitamin D levels are associated with higher blood pressure[7] and a higher rate of both incident hypertension[8] and cardiovascular disease[9;10] in observational studies. Previous intervention trials[11] have suggested that vitamin D may reduce blood pressure in selected groups of patients with hypertension; however no trials to date have evaluated the effects of vitamin D on resistant hypertension. Pathophysiological investigation suggests a link between low vitamin D levels and left ventricular hypertrophy, possibly mediated by parathyroid hormone[12;13], but very few studies have examined the effect of vitamin D analogues on left ventricular mass[14;15]. We therefore conducted a randomised controlled trial to test the effect

of intermittent high-dose vitamin D on 24 hour ambulatory blood pressure and left ventricular mass in patients with resistant hypertension.

Methods

Design and Participants

We performed a randomised, double-blind, placebo controlled, parallel group trial. Participants were recruited via cardiovascular medicine clinics and via primary care services in Tayside, Scotland. Participants were eligible for inclusion if they were aged 18 or over, had a supine office blood pressure of >140/90 mmHg, were on 3 or more antihypertensive medications (resistant hypertension), and had serum 25-hydroxyvitamin D (25OHD) levels <75nmol/L.

Participants were excluded if they had hypertension known to be due to a correctable underlying surgical or medical cause, had albumin-adjusted calcium levels of >2.60 mmol/L or <2.15 mmol/L, sarcoidosis, history of renal stones, or a previous clinical diagnosis of osteomalacia. Other exclusion criteria were liver function tests >3x upper limit of normal, estimated glomerular filtration rate by the Modified Diet in Renal Disease four variable equation of <40ml/min, metastatic malignancy, heart failure with left ventricular systolic dysfunction or known atrial fibrillation. Participants were excluded if already taking pharmacological vitamin D preparations (fish oils were permitted), were unable to give written informed consent, were pregnant, lactating, or were women of childbearing age without taking reliable contraception. Participants with contraindications to magnetic resonance imaging (MRI) scanning were permitted to enter the main study but were excluded from the MRI substudy. Written informed consent was obtained from all participants; ethical approval was given by Tayside Research Ethics committee (ref: 08/S1402/31). Clinical trials

authorisation was obtained from the UK Medicines and Healthcare Regulatory Authority (EuDRACT ref: 2008-002681-63) and the trial was prospectively registered with www.controlled-trials.com (ISRCTN63688695).All participants gave written informed consent, and the trial conformed to the principles of the Declaration of Helsinki.

Intervention

After completion of all baseline measurements including MRI if performed, participants were allocated the next sequentially numbered treatment pack. Packs contained either vitamin D3 (Vigantol oil, donated by Merck Serono KgAA) or identical placebo oil (Mygliol oil, used as the base oil for Vigantol). Medications were overlabelled and prepared by Tayside Pharmaceuticals (Dundee, UK) and were supplied in identical bottles with study number but no indication of group allocation. Allocation was therefore concealed from researchers and participants. After completion of assessments, a 5ml dose was administered by the study nurse at baseline, 2 months and 4 months to ensure 100% adherence. The total dose administered was therefore 300,000 units of vitamin D3 or placebo. All pre-study antihypertensive medications were continued.

Outcome measures

Outcomes were performed at 0, 2, 4 and 6 months by a research nurse blinded to study allocation. The primary outcome was between-group difference in mean 24 hour ambulatory systolic blood pressure at 6 months.

24 hour blood pressure was measured using Meditech ABPM-04 ambulatory blood pressure monitors and analysed using Cardiovisions data analysis software. Blood pressure was measured a minimum of every 30 minutes during the day (0600 to 2200 hrs) and a minimum

of every 60 minutes overnight (2200 hrs to 0600 hrs). The mean of the total 24 hour readings was used as the primary outcome measure. Office blood pressure was measured in the supine position after 5 minutes of rest using an OMRON HEM-705CP automated blood pressure cuff. Three consecutive readings were taken; the mean of the second and third readings were taken as the outcome measure. Fasting blood was drawn for measuring 25-hydroxyvitamin D, which was measured using the IDS radioimmunoassay (coefficient of variation 6.3%). Calcium, parathyroid hormone (PTH), creatinine, glucose and total cholesterol were measured according to standard protocols in the Department of Biochemical Medicine, NHS Tayside, Dundee, UK.

Echocardiography was performed at baseline on those participants willing and eligible to undertake the MRI substudy, using the method of Devereaux to estimate LV mass index[16]. Participants with left ventricular (LV) mass index of >110g/m^2 (male) or >95g/m^2 (female) were offered the opportunity to participate in the MRI substudy.

Cardiac MRI

Cardiac MRI was performed using a 3 Tesla Magnetom Trio scanner (Siemens, Erlangen, Germany). Following initial standard 'localiser' sequences, a series of 6mm short-axis (55 degree flip angle) 2D segmented cine ECG-gated breath-hold steady state free precession ('TrueFISP') images were acquired from the base to apex of the left ventricle to enable measurement of LV mass and wall thicknesses. Post-processing delineation of epicardial and endocardial borders (at end-diastole and end-systole) was performed using commercial cardiac evaluation software (Argus VB17, Siemens) by an experienced MRI physicist who was blind to the treatment allocation and not otherwise involved with the running of the study. Papillary muscles and trabeculae were routinely assigned to the LV blood pool if they

were identified as structurally distinct from the myocardial wall, but otherwise assigned to the myocardial mass. Only those image slices that displayed greater than 50% full-thickness myocardium were analysed. All datasets were evaluated twice over a time-course of one month in order to establish a mean (and corresponding repeatability evaluation) for the quantitative MRI parameters without the inclusion of segmentation learning effects. LV mass index was calculated by dividing LV mass by body surface area, calculated as the square root of ([weight in Kg x height in cm] / 3600). For measurement of left atrial end diastolic volume, 5mm multi-slice vertical long axis two chamber images were acquired from the lateral side of the left atrium to the atrial septum perpendicular to the plane of the mitral valve. The volume at atrial end-diastole contained by the atrial wall and the clearly delineated mitral valve was calculated. The left atrial appendage was included in the atrial volume measurements, and pulmonary vein structure was excluded wherever possible.

Asymptomatic hypercalcaemia (>2.60 mmol/L) was recorded as a prespecified adverse outcome, and no further doses of study medication were administered if this occurred. All adverse events were recorded at each study visit along with information on medication use and comorbid disease.

Statistical analysis

The trial was powered for an 8 mmHg fall in systolic blood pressure, based on data from previous trials using similar doses of vitamin D[17;18]. Assuming a standard deviation of change of 11mmHg, 31 patients per group (62 in total) would be required to detect this change with 80% power at alpha=0.05. To achieve this final evaluable sample size of 62 participants, we originally aimed to recruit a total of 74 patients to allow for a dropout rate of 20%, based on

previous similar studies in our department. For LV mass index, a change of 10 grams is regarded as clinically significant, and a total of 26 to 30 subjects is required to demonstrate a 10 gram change with 90% power at alpha=0.05[19].

Analyses were performed using SPSS version 18 (SPSS, Chicago, USA). Comparisons between continuous variables were performed using analysis of variance (ANOVA) at each timepoint. Categorical variables were compared using Pearson's chi-squared, or using Fisher's exact test when the contents of any cell was 5 or less. Repeated measures ANOVA was also undertaken to estimate the overall treatment effect using all available data for outcomes with more than two timepoints; for cardiac MRI, ANOVA was used to compare six month values between groups, adjusting for baseline values. Multiple imputation was used to address missing data for the primary outcome; 5 imputations were performed, using baseline and follow up blood pressure data, baseline age, sex and 25OHD level to generate imputed datasets. A sensitivity analysis was performed, excluding participants who changed their antihypertensive medication over the 6 month study period. A 2-sided p value of 0.05 was taken as significant for all analyses.

Results

Details of participant flow through the trial are given in Figure 1. 68 participants met the inclusion and exclusion criteria and were randomised into the study. Randomisation took place between January 2009 and February 2011. Baseline details for the 68 subjects randomised into the trial are shown in Table 1. All participants were of Caucasian ethnic background. 61/68 (90%) of participants underwent the 6 month visit. Recruitment was

terminated before the target number of participants was reached due in part to slow recruitment rates and in part due to the lower than anticipated dropout rate.

Table 2 shows the effect of the intervention on the 24 hour blood pressure and office blood pressure. No significant improvement in either 24 hour blood pressure or office blood pressure was seen with vitamin D supplementation at any timepoint; indeed the repeated measures analysis suggested a non-significant increase in blood pressure in the treatment group. Sensitivity analysis was performed using multiple imputation to address missing data for the primary outcome. The treatment effect for 24 hour ambulatory systolic blood pressure remained non-significant (+1mmHg, 95% CI -3 to +5), and the effect for 24 hour ambulatory diastolic blood pressure was of borderline significance (-3mmHg, 95% CI -6 to 0). A further sensitivity analysis was performed excluding the 9 participants (4 in vitamin D group, 5 in placebo group) who changed antihypertensive medication during the trial. In this analysis, little difference was seen in the results for 24 hour blood pressure (repeated measures systolic treatment effect 3mmHg, 95%CI -4 to 11, p=0.38; diastolic treatment effect 2mmHg, 95%CI -6 to 9, p=0.67) or for office blood pressure (repeated measures systolic treatment effect 3mmHg, 95%CI -4 to 10, p=0.38; diastolic treatment effect -3mmHg, 95%CI -8 to 2, p=0.30)

Adjusted repeated measures analysis of daytime ambulatory blood pressure change showed no effect of vitamin D (systolic treatment effect +1 mmHg, 95%CI -6 to 9; diastolic treatment effect -3mmHg, 95% CI -7 to +1); similarly, analysis of night-time ambulatory blood pressure change showed no significant treatment effect (systolic treatment effect +4 mmHg, 95%CI -3 to +12; diastolic treatment effect -1mmHg, 95% CI -6 to +3)

Table 3 shows the results of the cardiac MRI substudy. 37 patients progressed to MRI scanning at baseline; eight participants did not complete baseline MRI scans successfully, due to breathlessness (two participants), claustrophobia (three participants) and failure to fit into the scanner (three participants). A total of 29 patients therefore underwent successful baseline MRI scanning; 25 underwent follow-up scans at 6 months (11 in the treatment arm, 14 in the placebo arm). Left atrial images of sufficient quality for volumetric analysis at both baseline and follow up were available for 15 participants. Baseline end-diastolic volume and LV mass index were non-significantly lower in the vitamin D group. No difference in ejection fraction, left ventricular end-diastolic volume or left atrial end-diastolic volume was seen with treatment; LV mass index increased slightly with treatment, with this change reaching significance after adjustment for baseline blood pressure and 25OHD level.

Similar numbers of adverse events were noted in each group; 35 in the active treatment arm and 38 in the placebo group. No participant died during trial participation; one participant suffered a cardiovascular event in the active treatment group (angina) not requiring hospitalisation, and one suffered a cardiovascular event in the placebo group (myocardial infarction) requiring hospitalisation. No participant had serum calcium >2.60mmol/L at any timepoint. Details of adverse events are given in Supplementary Table 1

Discussion

This study failed to show any effect of high-dose, intermittent vitamin D supplementation on blood pressure, cholesterol, glucose or LV mass measured using cardiac MRI. This was despite the administration of relatively high doses of oral vitamin D (equivalent to 1800 units/day), with a substantial and sustained rise in 25OHD levels in the treatment group.

Several possibilities merit discussion to explain our findings. It is possible that the dose of vitamin D was insufficient to produce the required biological effect. Previous studies using similar doses in selected patients groups (e.g. those with type 2 diabetes) have however shown significant reductions in blood pressure; these studies included a proportion of patients with suboptimally treated hypertension[17;18]. Although the 25OHD levels fell short of the 75nmol/L level claimed by some commentators to be necessary for optimum health[20], no evidence exists to support a threshold effect of 25OHD level to produce beneficial vascular effects. Similarly, although the study was only of 6 months duration, reductions in blood pressure in previous studies have been seen within a few weeks of large oral doses of vitamin D. Reduction in LV mass on MRI has been demonstrated within 9 months with other vascular interventions, for instance by previous studies on allopurinol and on blood pressure reduction[21;22]. Our results are however consistent with the main findings of the PRIMO trial, which showed no effect of 48 weeks of paricalcitol therapy on LV mass in patients with advanced kidney disease[14]. Changes in left atrial volume may provide a more sensitive early measure of cardiac remodelling, and a substudy of PRIMO did find a reduction in left atrial volume with paricalcitol therapy[23]; we were unable to demonstrate this in the current study although the number of patients with usable left atrial volumetric information was low.

Another possibility is that only selected groups of patients at risk of vascular disease benefit from vitamin D supplementation. The effect of vitamin D on blood pressure in a previous meta-analysis was seen only in those studies where blood pressure was elevated at baseline[11] and patients with type 2 diabetes mellitus appeared to benefit more in terms of blood pressure reduction. Participants in the current study were all hypertensive at baseline, but few had diabetes. Participants were taking a wide range of antihypertensive agents, thus many of the

available biological pathways for blood pressure reduction may have already been engaged. Vitamin D has been posited to exert antihypertensive effects via effects on the renin-angiotensin-aldosterone system, either by direct inhibition of renin[24], or by angiotensin converting enzyme (ACE) inhibitor-like effects[25]. A high percentage of participants were taking ACE inhibitors, angiotensin receptor blockers and/or aldosterone antagonists; this may have served to obviate any further benefit of adding vitamin D. Another possibility is that those with different ethnic or genetic backgrounds may respond differently; a recent trial in black Americans showed a reduction in blood pressure with vitamin D; higher doses of vitamin D produced larger falls in blood pressure[26]. The lack of effect seen in this study does not exclude an effect in non-resistant or drug-naive hypertensive patients; such patients may still respond to manipulation of biological pathways by vitamin D that do not respond, or have already been utilised, in treated, resistant hypertensive patients.

Strengths of our study include the randomised, blinded design, the use of 24 hour blood pressure measurements, and the assessment of LV mass index – a key independent marker of vascular events and vascular death. Limitations include the relatively small study size, Caucasian study population, lack of follow-up beyond 6 months, and the obscuring effect of multiple treatments; an inevitable limitation when studying patients with resistant hypertension.

Perspectives

High-dose intermittent oral vitamin D3 therapy did not reduce blood pressure or LV mass in patients with resistant hypertension on multiple antihypertensive agents. Further research could focus on whether larger doses of vitamin D given for longer might be more effective,

or whether prespecified subgroups (e.g. those with diabetes or not taking renin-angiotensin system blockers) might still show reductions in blood pressure with vitamin D.

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Conflicts of interest

MDW and ADS have received grant support for other trials of vitamin D in cardiovascular disease from Diabetes UK, Chest Heart and Stroke Scotland, Scottish Government, ME Research UK, Heart Research UK

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Novelty and Significance:

1) What is new?
   - Trials of vitamin D supplementation to date have not focussed on patients with resistant hypertension; this placebo-controlled trial is the first to do so. There was no reduction in blood pressure or left ventricular hypertrophy after six months of high-dose, intermittent oral vitamin D3 supplementation
2) What is the significance?
   - Although observational data suggest a link between low vitamin D levels and higher blood pressure, trial results to date have been inconsistent. These results do not support the use of vitamin D as a therapy for this difficult to treat group of patients.
3) Summary:
   - This dose and duration of vitamin D was not effective at reducing blood pressure or left ventricular mass in this population of patients with resistant hypertension.

Figure legends:

Fig 1. Participant flow through trial

Table 1. Baseline details of randomised participants

Parameter	Active (n=34)	Placebo (n=34)	P
Age (years) (SD)	65.1 (9.4)	61.4 (12.7)	0.18
Male Sex (%)	22 (65)	22 (65)	1.0
Mean office systolic blood pressure (mmHg) (SD)	153 (11)	155 (14)	0.51
Mean office diastolic blood pressure (mmHg) (SD)	82 (10)	86 (9)	0.06
Mean 24 hr systolic blood pressure (mmHg) (SD)	138 (18)	137 (13)	0.76
Mean 24 hr diastolic blood pressure (mmHg) (SD)	76 (8)	78 (9)	0.38
Mean daytime systolic blood pressure (mmHg) (SD)	142 (18)	140 (13)	0.72
Mean daytime diastolic blood pressure (mmHg) (SD)	78 (9)	81 (9)	0.16
Mean nighttime systolic blood pressure (mmHg) (SD)	134 (20	128 (16)	0.18
Mean nighttime diastolic blood pressure (mmHg) (SD)	71 (8)	73 (10)	0.53
Previous myocardial infarction (%)	5 (15)	3 (9)	0.48
Angina (%)	7 (21)	5 (15)	0.54
Peripheral vascular disease (%)	1 (3)	0 (0)	1.0
Stroke / TIA (%)	3 (9)	4 (12)	0.26
Diabetes mellitus (%)	9 (27)	8 (24)	1.0
Current smoker (%)	3 (9)	1 (3)	0.61
BMI (Kgm^{-2}) (SD)	31.4 (5.3)	32.0 (5.5)	0.61
LV mass index by echocardiography (gm^{-2}) (SD)	140 (40)	140 (23)	0.97
Median number of antihypertensives (IQR)	3.5 (2)	3 (1)	0.54
On ACE inhibitor (%)	14 (42)	17 (50)	0.47
On ARB (%)	17 (50)	17 (50)	1.0
On alpha blocker (%)	19 (54)	12 (35)	0.09

On beta blocker (%)	13 (38)	14 (42)	0.80
On calcium channel blocker (%)	22 (65)	28 (84)	0.10
On thiazide (%)	20 (59)	17 (50)	0.47
On aldosterone antagonist (%)	8 (24)	11 (32)	0.42
On any diuretic (%)	29 (85)	30 (88)	0.72
25OHD (nmol/L) (SD)	41 (14)	42 (18)	0.85
Adjusted calcium (mmol/L) (SD)	2.29 (0.08)	2.32 (0.06)	0.10
PTH (pmol/L) (SD)	5.6 (2.7)	5.2 (2.2)	0.44
Glucose (mmol/L) (SD)	6.8 (3.0)	6.1 (2.0)	0.31
Cholesterol (mmol/L) (SD)	4.6 (0.8)	4.6 (1.1)	0.90

TIA: Transient ischaemic attack

BMI: Body mass index

LV: Left ventricular

ACEi: Angiotensin converting enzyme inhibitor

ARB: Angiotensin receptor blocker

25OHD: 25-hydroxyvitamin D

PTH: Parathyroid hormone

Table 2. Effect of vitamin D supplementation on mean 24 hour ambulatory blood pressure and office blood pressure

24 hour ambulatory blood pressure						
Timepoint	Vitamin D	Placebo	P	Vitamin D	Placebo	P
	Systolic BP (mmHg)(SD)			*Diastolic BP (mmHg)(SD)*		
Baseline	138 (18)	137 (13)	0.76	76 (8)	78 (9)	0.38
2 mths	136 (15)	134 (13)	0.55	75 (8)	79 (9)	0.09
4 mths	137 (17)	130 (9)	0.25	73 (8)	79 (10)	0.10
6 mths	136 (14)	130 (14)	0.12	76 (8)	78 (9)	0.28
Unadjusted RM*	4 (-3 to 11)		0.20	-2 (-6 to 2)		0.29
Adjusted RM* †	3 (-4 to 11)		0.33	-2 (-7 to 2)		0.26
Office blood pressure						
	Vitamin D	Placebo	P	Vitamin D	Placebo	P
	Systolic BP (mmHg)(SD)			*Diastolic BP (mmHg)(SD)*		
Baseline	153 (11)	155 (14)	0.51	82 (10)	86 (9)	0.06
2 mths	154 (18)	150 (12)	0.40	82 (10)	83 (11)	0.66
4 mths	152 (14)	147 (13)	0.21	81 (11)	82 (10)	0.64
6 mths	151 (19)	146 (16)	0.35	82 (8)	80 (11)	0.40
Unadjusted RM*	4 (-2 to 10)		0.24	-3 (-7 to 1)		0.18
Adjusted RM*	4 (-3 to 10)		0.25	-3 (-7 to 1)		0.18

†

*Repeated measures treatment effect. †Adjusted for baseline 25OHD level

Table 3: Cardiac MRI measures

Timepoint	Active (n=11)	Placebo (n=14)	p
Baseline EF (%) (SD)	72.3 (6.0)	71.5 (6.5)	0.74
6 month EF (%) (SD)	70.6 (3.7)	70.0 (8.1)	0.79
Unadjusted treatment effect (95% CI)	0.2 (-4.7 to 5.2)		0.92
Adjusted* treatment effect (95% CI)	2.0 (-3.1 to 7.1)		0.43
Baseline LVMI (gm^{-2}) (SD)	57 (13)	62 (16)	0.58
6 month LVMI (gm^{-2}) (SD)	58 (13)	61 (16)	0.58
Unadjusted treatment effect (95% CI)	2 (-1 to 5)		0.26
Adjusted* treatment effect (95% CI)	4 (0 to 7)		0.04
Baseline LVEDV (cm^3) (SD)	140 (32)	155 (26)	0.21
6 month LVEDV (cm^3) (SD)	136 (26)	154 (41)	0.22
Unadjusted treatment effect (95% CI)	-3 (-22 to 16)		0.73
Adjusted* treatment effect (95% CI)	-8 (-29 to 13)		0.45
	n=7	n=8	
Baseline LAEDV (cm^3) (SD)	116 (41)	88 (16)	0.10
6 month LAEDV (cm^3) (SD)	113 (35)	95 (15)	0.22
Unadjusted treatment effect (95% CI)	-3 (-20 to 13)		0.67
Adjusted* treatment effect (95% CI)	-3 (-30 to 22)		0.77

*adjusted for baseline systolic BP and 25OHD level

EF: Ejection fraction
LVMI: Left ventricular mass index

LVEDV: Left ventricular end diastolic volume
LAEDV: Left atrial end diastolic volume

Table 4. Metabolic outcomes

Parameter	Repeated measures treatment effect (95% CI)	p
25OHD (nmol/L)	22 (17 to 28)	<0.001
Adjusted calcium (mmol/L)	-0.01 (-0.04 to 0.02)	0.54
PTH (pmol/L)	-0.8 (-1.5 to 0.0)	0.05
Fasting glucose	0.0 (-0.8 to 0.8)	0.99
Total cholesterol	0.1 (-0.2 to 0.3)	0.57

25OHD: 25-hydroxyvitamin D

PTH: Parathyroid hormone